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Comparative Aspects of Pesticide Metabolism in Plants and Animals

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Pesticide chemicals are an important component of modern agriculture. Through their use, plants and animals are exposed to pesticides directly and indirectly from transport through soil, water, and other environmental components. Pesticide chemicals which are absorbed by plants and animals undergo extensive biotransformation. Lipophilic compounds are converted to polar metabolites through a variety of microsomal and extramicrosomal reactions in plants and animals. Generally, biotransformations are qualitatively similar in both systems. However, there are important quantitative rate differences in metabolism which often determine the balance between activation and deactivation of a pesticide. Furthermore, there are qualitative differences in conjugative mechanisms in plants and animals. Animals through an efficient excretory system eliminate transformation products via the urine and feces. Since efficient excretory systems are absent in plants, terminal degradation products are stored as conjugates and/or derivatives which may be incorporated into the plants themselves.

Metabolic transformations of selected pesticides illustrating various types of reactions in plants and animals are discussed.

Pesticide chemicals are an integral component of modern farming. Energy conservation measures, precision planting, and provisions for optimal production of food and fiber crops are intimately interwoven with the use of pesticide chemicals. The need for using pesticide chemicals to increase food production on a global scale was recently reviewed by Goring (*1*).

Pesticide chemicals are primarily used on food and fiber crops, in soils, on pasture, and on forest land; to a lesser extent they are used to protect human and animal health.

Plants are the major ultimate recipients of these chemicals, either from direct application, soil uptake, or atmospheric drift. Pesticides may reside on the surface of plants or by virtue of their lipophilicity they may penetrate the cuticle of leaves, fruits, stems, roots, and seeds (*2*).

Plant foliage is a major route of absorption of pesticides. Hull (*3*) described the relation between leaf structure and absorption of xenobiotics by plants. Once deposited on the arboreal portions of the plant, the chemical is absorbed by cuticular waxes where it can be stored or can penetrate into plant cells. In the process of transport across the primary cell wall, the secondary wall, and the plasmalemma (*4*), the chemical encounters several hydrolytic enzymes which are capable of hydrolyzing xenobiotics such as esters, amides, and others prior to penetration into the protoplast. Pesticides can be transported short distances intercellularly through plasmadesmata (*5*) and long distances within the vascular elements of plants. The latter is considered systemic transport. According to Crafts and Crisp (*6*), substances transported in the living protoplasm of plants via the phloem are symplastic. Only a few pesticides such as aminotriazole, maleic hydrazide, 2,4-D type esters, and certain plant growth regulators move symplastically. Most systemic insecticides such as demeton, dimethoate, disulfoton, and phorate, while considered systemic (*2*), indeed move passively in the nonliving xylem elements and are classified as apoplastic (*6*).

Another major route of absorption of soil applied pesticides is through the root cap and root hairs (*7*). Pesticides may be transformed in the roots and then transported upwards in the apoplast.

Unlike animals, where primarily unchanged compounds are absorbed directly via the oral, dermal, and inhalation routes (*8*), pesticides, prior to uptake by plants, may undergo transformations due to photolytic and/or hydrolytic action on plant sur-

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faces or microbial action in the soil. The contribution of these factors to transformations observed in the plant require further research under carefully controlled conditions.

Both animal and plant cells are eucaryotic with very similar structural features (*9*). However, plant cells have a cell wall whereas animal cells have a glycoproteinaceous plasma membrane. Furthermore, higher plant cells contain chloroplasts which serve as the site of transformation of radiant energy to chemical energy. Both animal and plant cells contain a network of membranes and vesicles called the endoplasmic reticulum. Upon differential centrifugation (100,000*g*), a pellet is obtained designated as the microsomal fraction (microsomes). Many important enzymes responsible for biotransformation of xenobiotics are included in this fraction. Additional metabolizing enzymes are present in various other subcellular components including the cytosol fraction (*10*).

In vertebrates, metabolizing enzymes are found mainly in the endoplasmic reticulum of the liver (*8*) and to a lesser extent, the lung, kidney, digestive tract, brain, and skin. In insects, microsomal enzymes are primarily associated with gut tissues and/or fat body (*11*). Relatively little information is available on localization of microsomal enzymes in plants. Microsomal preparations have been obtained from leaves, meristematic tissue, and other tissues. In one of the few reported instances, Frear and coworkers (*12*) obtained a well defined plant microsomal enzyme preparation from etiolated cotton seedling hypocotyls which catalyzed the oxidative *N*-demethylation of 1,1-dialkylated phenylureas. However, our knowledge of microsomal enzymes in plants is still very fragmentary due to the very limited number of successful *in vitro* preparations of these enzymes (*7*).

Important biotransformation reactions occurring in animals and higher plants are summarized in Table 1. This outline is largely based on several recent reviews (*7, 8, 10–17*). Selected examples of metabolic transformations are taken from the pesticide biochemistry literature. The types of reactions are divided into those classified as microsomal transformation, these being primarily oxidations, and those reactions classified as nonmicrosomal or extramicrosomal transformations. In some instances, such as hydrolysis, transformations may also be catalyzed by microsomal enzymes.

A study of the current literature leads us to conclude that indeed the state of the art as it pertains to characterization of metabolizing enzymes is much more advanced in the animal than in the plant field. However, in many instances the biotransformation products arising from pesticide metabolism in animals and plants are similar or the same. Thus, we may infer that the same or similar enzymes catalyze mostly the same reactions in plants and animals.

As a final step in the complex events in biotransformation reactions, the xenobiotic molecule may undergo conjugation reactions in animals and plants. Often this serves as the final elaboration in the long chain of detoxification reactions. The types of conjugations encountered in animals and plants are listed in Table 2. As already mentioned, microsomal and/or nonmicrosomal biotransformations provide the modified xenobiotic molecule or its modified scission products with reactive groupings, such as $-OH$, $-SH$, $-COOH$, or $-NH_2$ which serve as functional sites for further elaboration as conjugates. The conjugated species primarily facilitate excretion in animals and immobilization in plants.

As in animals, higher plants have a great diversity of biochemical processes and enzymes leading to formation of the types of conjugates shown in Table 2. While glucuronide and sulfate conjugation is widespread in animals, in plants these, at best, constitute exceptional reactions. On the other hand, glycoside conjugation is widespread in plants and only of minor importance in mammals (*19*). Perhaps due to low excretion potential, plants can further elaborate sugar conjugates into gentiobiosides (disaccharides) (*7*). Amino acid conjugation and acylation are common conjugation pathways in mammals and plants. Glutathione (GSH) conjugation is ubiquitous in higher animals and plants. However, mammals can further modify the cysteine derivative of the initially formed glutathione conjugate by means of acylation in the kidney to yield a mercapturate as the terminal product of glutathione conjugation which is subsequently excreted in urine. Acid derivatives of pesticide metabolites have not been isolated from plants (*7*); however, in a few instances and by means of an unknown mechanism, the cysteine conjugate is converted to a lanthionine conjugate. Perhaps the plant utilizes this mechanism as in the case of glycosides to form conjugates which can be further polymerized and incorporated into structural plant components as an alternative to excretion (*20*).

In the following portion of this review, selected examples of metabolic transformations in plants and animals will be discussed to illustrate some of the types of biotransformation reactions shown in Tables 1 and 2.

Dehydrochlorination and Dechlorination

DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloro-

Table 1. Major biotransformation reactions of pesticides in animals and plants.

Type of reaction	Examples[a]
Microsomal	
Aromatic C-H hydroxylation	Fonofos, carbaryl
Aliphatic C-H oxidation	DDT→Dicofol, pyrethroids
N-Oxidation	Nicotine→Nicotine-1'-oxide
S-Oxidation	Disulfoton, carbophenothion, Phorate, thiocarbamates
Desulfuration	Parathion→Paraoxon
O- and *S*-Desalkylation	Chlorfenvinphos, Gardona, methoxychlor
GSH, GSH-*S*-transferase	Fenitrothion, fluorodifen
N-Desalkylation	Monuron, dicrotophos
Epoxidation	Cyclodienes, IGRs
Reduction (sulfoxide)	Carbophenothion sulfoxide, Fensulfothion sulfoxide
Reduction (nitro)	Parathion→Amino parathion
Hydrolysis	Pyrethroids
Nonmicrosomal	
Phosphotriester hydrolysis	Various OPs
Carboxylester hydrolysis	Malathion→Monoacid
Carboxylamide hydrolysis	Dimethoate→Acid, propanil
Nitrile hydrolysis	2,4-D-Alkanenitrile→Carboxy acid
Dehydrohalogenation	DDT→DDE
Dechlorination	Atrazine
Epoxide hydrase	IGRs, cyclodienes
Nitroreductase	Parathion→Amino parathion

[a] Where available common names of pesticides are used as listed in Martin and Worthing (*18*).

Table 2. Major conjugation reactions involving pesticides in animals and plants.

Type of conjugation	Remarks
Glucuronide formation	Prevalent in vertebrates
Glycoside formation	Prevalent in plants and insects
Glutathione conjugation	Atrazine, thiocarbamates, captan
Mercapturates	Animals only
Cysteine conjugation	Plants, animals
Glycine conjugation	Plants, animals
Other amino acid conjugation	Plants, animals
Sulfate conjugation	Prevalent in animals, rare in plants
O and *S*-Methylation	Animals, plants
Thiocyanate formation	Animals, plants
N-Acetylation	Animals (DNOC), plants (desmedipham)

ethane, is one of the most studied insecticides. It is still widely used for vector control in malaria programs world-wide. However, in many parts of the world, including the USA and USSR, it was banned because of its environmental persistence and accumulation in living organisms. Unlike most pesticides where lipophilic species are converted to more polar derivatives, initial biotransformation of DDT in animals and plants involves a dehydrochlorination reaction catalyzed by DDT dehydrochlorinase (*11*) which yields the nonpolar and persistent metabolite DDE (Fig. 1). Harrison, et al. (*21*) demonstrated that leaf tissues convert DDT to DDE within one week after treatment. After longer exposure DDT was also reductively dechlorinated to DDD; the latter was further hydroxylated and oxidized to dichlorobenzophenone. In barley DDT remained largely unmetabolized (*22*). In stored wheat grain DDT remained largely unchanged with only minor conversion to DDE (*23*). The reductive dechlorination of DDT to DDD is most likely of bacterial origin; those associated with plants (*24*). It is associated with anaerobic peroxidation of unsaturated fats by nonporphyrin enzymes. Oxidation of DDT to DDA and the resultant conjugates are the major mammalian metabolites excreted in urine (*15*, *24*). An unusual biotransformation in insects was reported by Tsukamoto and later by Agosin and co-workers (*24*), who found extensive metabolism of DDT to the acaricide, dicofol (Kelthane).

The foregoing biotransformation reactions (Fig. 1) also illustrate the principle of metabolic bioacti-

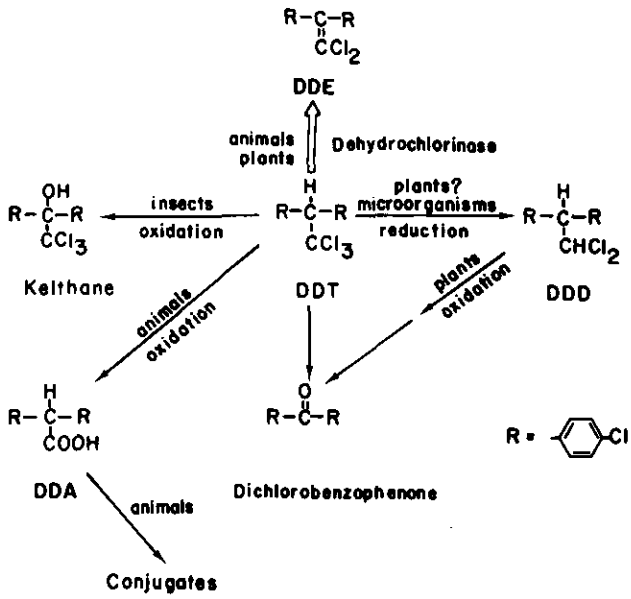


FIGURE 1. Biotransformation of DDT in plants, animals, and microorganisms.

vation since DDD is also an active insecticide and dicofol is an acaricide.

The well known herbicide, atrazine, 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine, is detoxified by several routes including a dechlorination reaction in roots of corn which renders the herbicide nontoxic to the plant. The reaction is apparently nonenzymatic and is mediated by a natural corn constituent, 2,4-dihydroxy-7-1,4-benzoxazin-3-one (*25*). However, the more important detoxification reaction in tolerant plants involves a nucleophilic displacement reaction involving removal of the chlorine and substitution by glutathione. In the rat this conjugate is further metabolized to a mercapturic acid derivative (*26*). The latter is readily excreted in urine while the plant conjugate may undergo further transformations and incorporation into plant components.

Desulfuration and Thioether Oxidation

Organophosphorus ester insecticides (OPs) constitute one of the major classes of pesticides in use today. Most OPs are thiono esters which are acutely less toxic than their corresponding oxons, the latter being potent cholinesterase inhibitors. Desulfuration of the P→S moiety to P→O is catalyzed by microsomal oxidases in mammals and insects (*11*).

Experiments with $^{18}O_2$ and $H_2{}^{18}O$ and rat liver microsomes have shown that the P→O oxygen is derived from air via a postulated oxygenated intermediate (Fig. 2) which cleaves to the corresponding oxon and a reactive sulfur atom. The presence of the oxygenated intermediate was proposed independently by McBain et al. (*27*) for fonofos and by Ptashne et al. (*28*) for parathion. According to the scheme shown (Fig. 2), the oxygenated intermediate of fonofos rearranges to the oxon, or it hydrolyses yielding the cleavage products EOP and ETP. The acute oral toxicity values in rats show that the oxon is fivefold more toxic than fonofos and is a potent inhibitor of bovine erythrocyte acetylcholinesterase (AChE) (*29*). In plants, the mechanism of desulfuration has not been determined to date. However, oxons arising from parent thiono OPs have been recovered from plants. They are formed either photolytically on plant surfaces or by action of oxidative plant enzymes. Knaak et al. (*30*) demonstrated the conversion of parathion to paraoxon using a plant peroxidase preparation from horseradish and bean hypocotyls. Malathion also undergoes desulfuration in plants giving rise to malaoxon (*31*). Again, as with parathion, the resulting oxon is an "activation" product more toxic to mammalian species and concomitantly a more potent AChE inhibitor (Table 3). Usually these oxons are either sparingly formed or rapidly degraded to terminal detoxification products.

Thioether oxidation or sulfoxidation in animals and plants is an important biotransformation pathway involving a number of important OP and carbamate insecticides. Thioether oxidation occurs with the carbamate insecticide, methiocarb, in bean plants, in rat liver microsomes, and by housefly enzymes (*32*). Similarly, sulfoxidation of the oxime carbamate, aldicarb, is a major metabolic reaction in mammals, plants, and insects (*32*).

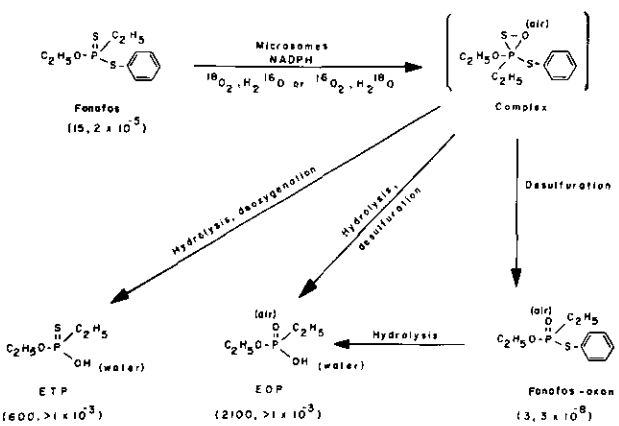


FIGURE 2. Bioactivation and inactivation mechanism of fonofos. Values in brackets denote rat oral LD_{50} (mg/kg), (I_{50}, *M*) bovine erythrocyte AChE. Data from Menn et al. (*29*).

Table 3. Toxicity and acetylcholinesterase inhibition values for parathion, malathion, and their corresponding oxons.[a]

Compound	Rat oral LD_{50}, mg/kg	AChE inhibition I_{50}, M
Parathion	3.3	1×10^{-4}
Paraoxon	1.4	6.6×10^{-9}
Malathion	2,600	2.9×10^{-3}
Malaoxon	308	7.0×10^{-7}

[a] Data from Eto (*13*).

Thioether oxidation of OP insecticides in animals and plants is well documented in the literature (*7, 11, 13, 24, 33*). Early landmark studies were published by March et al. (*34*) and Metcalf et al. (*35*). They demonstrated that the insecticides demeton (Systox), phorate (Thimet), and disulfoton (Dithio-Systox) were sulfoxidized to the corresponding sulfoxides and sulfones in the mouse, American cockroach, intact cotton leaves, lemon fruit, bean, and alfalfa plants. In the process of biotransformation, the parent thiono compounds were also desulfurated and gave rise to the corresponding sulfoxides and sulfones.

Thioether oxidation of OP insecticides is particularly relevant to control of plant sucking insects, since it renders these insecticides systemic (apoplastic) in plants. Acute toxicity of the sulfoxidized species is not necessarily enhanced in comparison to the parent OP (*13*). This is possibly due to increased lability and subsequent degradative metabolism.

Another example of thioether oxidation relates to the oxidative metabolism of carbophenothion in lettuce plants and orange trees (*29*). As shown (Fig. 3), carbophenothion was oxidized to the sulfoxide (II), sulfone (III), and the sulfoxide (V), and sulfone (VI) of the oxon (IV). Trace amounts of these sulfoxidized derivatives were detected in the citrus foliage and fruit peel but not in the juice and pulp. As reported for other thioether OPs (*13*), anticholinesterase activity generally increased as a function of increased sulfoxidation. However, none of the intact, oxidized metabolites of carbophenothion were detected in living rats. Apparently biotransformation is more rapid and extensive in animals. Indeed, only cleavage products involving the *p*-chlorophenylthio moiety of carbophenothion were recovered in rat urine (*29, 36*).

Oxidation

Oxidation reactions play a major role in the biotransformation of pesticides in animals (*11*) and plants (*7, 33, 37*). Indeed, most biotransformation reactions are oxidative including desulfuration and

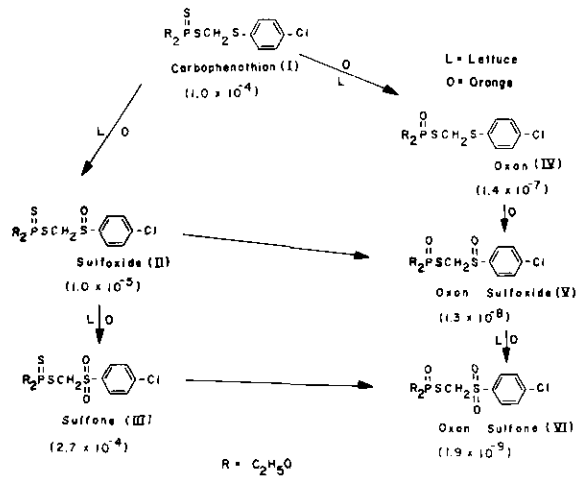


FIGURE 3. Oxidative metabolism of carbophenothion in lettuce (L) and orange (O). Values in brackets denote human plasma ChE I_0 molar concentration values. Data from Menn et al. (*29*).

thioether oxidation (already discussed), aliphatic and aryl hydroxylation, epoxidation, and *N*- and *S*-oxidation. Examples of some of these oxidative biotransformations are further detailed in the following discussion.

Thiocarbamate herbicides are an important group of nonpersistent preemergent herbicides primarily effective in controlling grassy weed species. Recent studies by Hubbell and Casida (*38*) have established the metabolic fate of EPTC (*S*-ethyl *N,N*-dipropylthiocarbamate) and butylate (*S*-ethyl *N,N*-diisobutylthiocarbamate) in rats and corn. Microsomal oxidation of the *S*-alkyl moiety to the corresponding sulfoxides in the rat is the key biotransformation reaction predisposing the herbicide to the subsequent biotransformations shown in Figure 4. Furthermore, these sulfoxides, under laboratory and greenhouse conditions, display enhanced herbicidal activity relative to their respective thiocarbamates (*39*), but they are not more toxic to mammals (*38*). Further oxidation to the sulfones is theoretically likely to occur. However, they are hydrolytically unstable and not likely to persist *in vivo* (*40*). The thiocarbamate sulfoxides are cleaved at the carbonyl group by the glutathione (GSH) and GSH-*S*-transferase system forming a GSH conjugate which is further cleaved by peptides to the cysteine conjugate. These biotransformations occur in both rats and corn. However, in rats the cysteine conjugate is modified further since the following metabolites were found in rate urine: the mercapturic acid conjugate; its *N*-dealkylation product, *S*-(*N*-alkylcarbamoyl)-*N*-acetylcysteine; the further

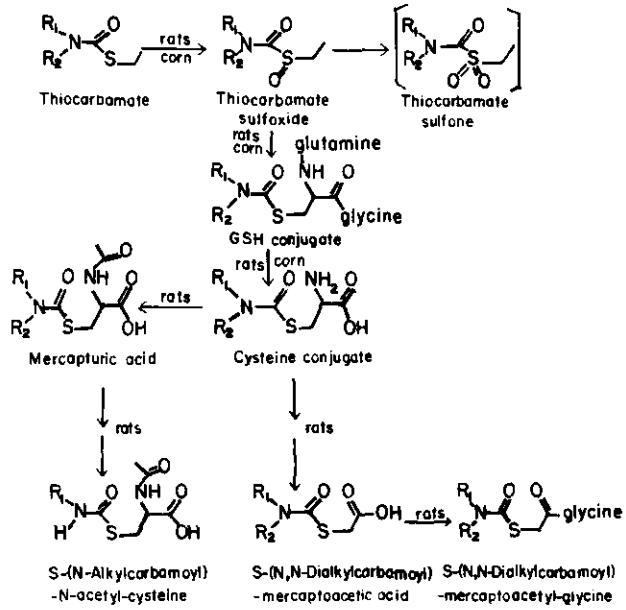


FIGURE 4. Metabolism of thiocarbamate herbicides in the rat and corn; R_1, R_2 = propyl or isobutyl. Adapted from Hubbell and Casida (*38*).

degradation product, *S*-(*N*,*N*-dialkylcarbamoyl)-mercaptoacetic acid; and a portion of the latter conjugated with glycine to form *S*-(*N*,*N*-dialkylcarbamoyl) mercaptoacetylglycine.

The facile degradation of the thiocarbamates helps explain their nonpersistency in the environment. All of the identified metabolites are detoxification products, less toxic than the parent compounds (*38*). In conjunction with thiocarbamate metabolism studies, Lay et al. (*41*, *42*) demonstrated a biochemical mechanism which enhances detoxification of thiocarbamate sulfoxides in corn plants. Studies have demonstrated that *N*,*N*-diallyldichloroacetamides (*43*) elevate GSH and GSH-*S*-transferase content in the corn plant resulting in more rapid degradation of the thiocarbamate sulfoxides through carbamoylation of GSH and formation of *S*-carbamyl-GSH conjugates (Fig. 5). In susceptible plant species, such as oats, the GSH-*S*-transferase level is low and is not enhanced by the dichloracetamide antidotes and consequently they remain susceptible to the thiocarbamate herbicides (*41*, *42*).

Thioaryl moieties may act as leaving groups in the course of detoxification reactions involving pesticides containing this group. In conjunction with metabolic studies in plants and animals of the insecticide fonofos which contains a thioaryl moiety, McBain and Menn (*44*) demonstrated that thiophenol was rapidly methylated and sulfoxidized as the primary steps in its detoxification in the rat. This finding served as a model for plant metabolism studies with Irish potatoes using ^{14}C-phenyl fonofos (*45*) and rat metabolism studies (*46*). Figure 6 depicts the comparative metabolic pathway of fonofos in the animal and plant. As shown, a similar detoxification pathway was established in both systems, involving desulfuration, cleavage to transient thiophenol (PhSH) and thioanisole (PhSMe), and subsequent sulfoxidation to phenyl methyl sulfone ($PhSO_2Me$). In the rat $PhSO_2Me$ is extensively 3- and 4-hydroxylated and excreted primarily as acid labile conjugates. Although large amounts of water-soluble products were recovered from polar

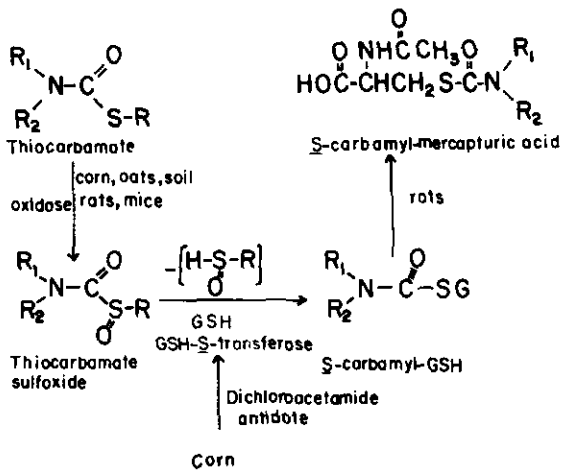


FIGURE 5. Enhancement of thiocarbamate sulfoxide detoxification in corn plants via elevated levels of GSH and GSH-*S*-transferase induced by dichloroacetamide antidotes. Adapted from Lay et al. (*41*).

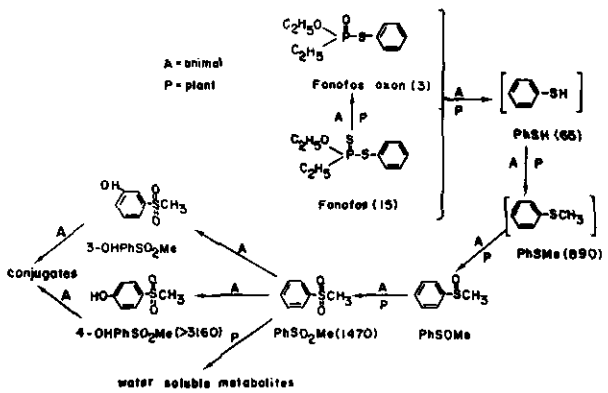


FIGURE 6. Comparative metabolic pathway of fonofos in the rat and potato plant. Values in brackets denote acute oral rat LD_{50} values in mg/kg. Based on data from McBain (*45*, *46*).

plant extracts, they did not correspond to the hydroxylated urinary metabolites. However, acid hydrolysis or enzymatic digestion released phenylmethyl sulfoxide (PhSOMe) and sulfone ($PhSO_2Me$) suggesting that the unknown polar plant metabolites were derived from these precursors. Significantly, only trace amounts of fonofos-oxon were recovered from rat urine and the plant suggesting that it was either sparingly formed *in vivo* or rapidly cleaved to the products shown (Fig. 6). The toxicity values shown (Fig. 6) for the transformation products of fonofos in plants and animals clearly indicate the overall potential of plants and animals to detoxify xenobiotics along similar biotransformation pathways.

Direct oxidation of the sulfur of the thioaryl moiety of the experimental insecticide, N-2596, *O*-ethyl-*S*-4-chlorophenyl ethanephosphonodithioate, was reported in the rat (*47*) and in corn plants (*48*). The comparative metabolic pathway in the rat following oral dosing with [^{14}C]phenyl-N-2596 and via root uptake in the growing corn plant is outlined in Figure 7. In the plant and in the rat N-2596 undergoes rapid cleavage. Only trace amounts of N-2596 (0.3%) and its oxon (0.4%) were recovered in the plant and none were found in rat urine. The radiocarbon in urine accounted for 80% of the dose. Although there is no direct evidence indicating the extent to which the oxon might be formed, and since the thioaryl cleavage products could arise from either N-2596 or its oxon, it may be assumed that the latter, if formed, is rapidly cleaved in both animals and plants. Qualitatively the subsequent biotransformation steps involving the 4-chlorothiophenol (4-ClPhSH) are similar in the animal and plant including the *S*-methylation and sulfoxidation reactions. However, *S*-methylation, and ring hydroxylation and subsequent sulfate and glucuronide conjugation predominate in the animal. In contrast, no conjugation was detected in the plant and 4-chlorobenzene sulfonic acid (4-$ClPhSO_3H$) was recovered as the major terminal metabolite. The latter was also recovered as a minor urinary metabolite. The foregoing data show that direct oxidation to the sulfonic acid is the preferred pathway in the plant, whereas oxidation on the ring (hydroxylation) is the major pathway in the rat. As shown with fonofos, the biotransformation of N-2596 in animals and plants is a detoxification pathway as suggested by the animal toxicity and AChE inhibition data shown in Table 4.

Reduction

Reduction of aromatic nitro compounds to aniline derivatives catalyzed by nitroreductases in the microsomal and soluble fractions of liver preparations are known to occur (*15*). These transformations occur with the use of $NADPH_2$ or $NADH_2$ under anaerobic conditions. Lichtenstein et al. (*49*) have also shown that parathion and paraoxon were re-

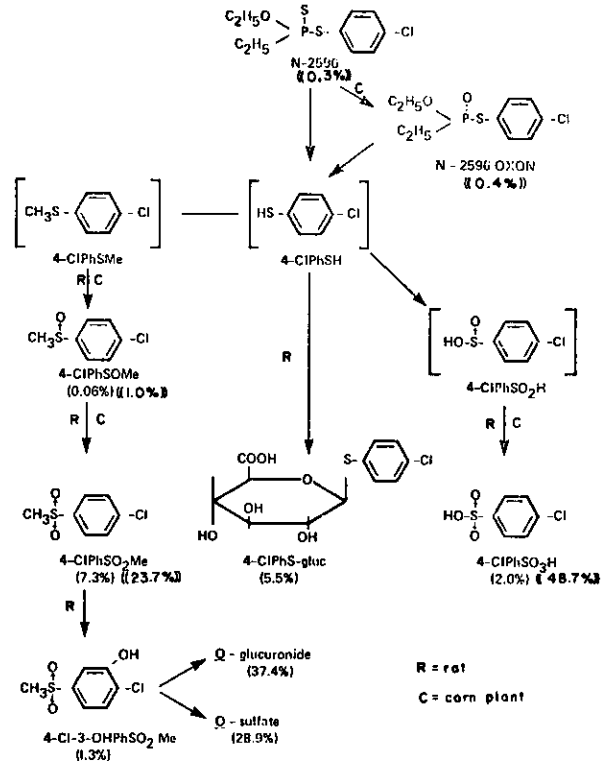


FIGURE 7. Comparative metabolism of N-2596 in the rat and corn plant. Percent values in brackets are for distribution of radiocarbon in urine; in double brackets, percent of total radiocarbon recovered in plants. Adapted from Miaullis et al. (*47*).

Table 4. Toxicity and acetylcholinesterase inhibition values for N-2596, metabolites, and related compounds.[a]

Compound	Rat oral LD_{50}, female (95% confidence range), mg/kg	Bovine erythrocyte acetylcholinesterase inhibition I_{50}, M[b]
N-2596	3.23 (2.2-4.6)	2×10^{-5}
N-2596 Oxon	5.8 (4.3-7.9)	2×10^{-9}
4-ClPhSH	316 (216-463)	$>1 \times 10^{-3}$
4-ClPhSMe	>500	$>1 \times 10^{-3}$
4-ClPhSOMe	>500	$>1 \times 10^{-3}$
4-$ClPhSO_2Me$	>500	$>1 \times 10^{-3}$
4-Cl-3-$OHPhSO_2Me$	>500	$>1 \times 10^{-3}$
4-$ClPhSO_2H$	>500	
4-$ClPhSO_3H$	>500	

[a] Data from Miaullis et al. (*47*).

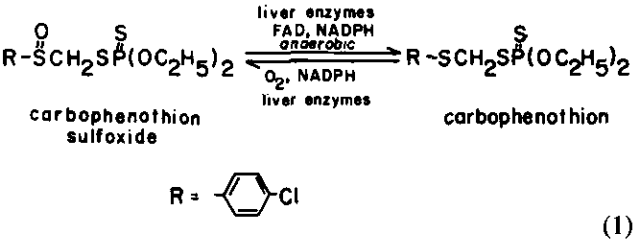
[b] I_{50}, molar concentration inhibiting 50% of the enzyme in 10 min at 37°C.

duced to their respective amino derivatives under aerobic conditions by 105,000 and 500,000 *g* rat liver supernatants, and to a lesser degree by microsomal fraction.

Several reductive transformations have been shown to occur in plants (*7*). The herbicidal nitrobenzoic acid derivative, dinoben, was reduced to the amino derivative in soybean roots (*50*). Suzuki and Uchiyama (*51*) anaerobically incubated parathion with spinach leaf homogenates, NADP, FAD, and G-6-P cofactors. Parathion via an unstable nitroso derivative was reduced to hydroxylamino parathion which was further reduced to amino parathion. Biswas and Hamilton (*52*) reported that the reduction of an *N*-demethylated nitrophenyl metabolite of the herbicide trifluralin to the amino derivative was mediated by a crude enzyme preparation from sweet potatoes. The same reduction occurred in carrot roots grown in soil treated with trifluralin (*53*).

Frehse (*54*) reported on an apparent sulfoxide reduction in plants. Fensulfothion, the reduced form of fensulfothion sulfoxide, was recovered from field treated bean plants. However, it was not clear whether the reduction was solely due to reductive plant enzymes or to action of microorganisms associated with the plant.

An unusual sulfoxide reduction in rats related to OP insecticide detoxification was described by DeBaun and Menn (*36*), who have shown that carbophenothion sulfoxide was reduced to carbophenothion in the living rat and in an *in vitro* system containing rat liver enzymes, NADPH and FAD [Eq. (1)]. Previously, it was well accepted that



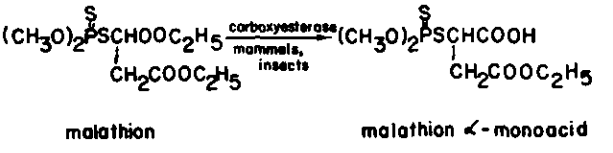
(1)

sulfoxidation of a thioether moiety of an OP insecticide represented an irreversible intoxication reaction (lethal synthesis) (*24*). The finding of *in vitro* and *in vivo* reduction of carbophenothion sulfoxide (*36*) suggests that sulfoxidation may be reversible in other OP insecticides and may represent a more general detoxification pathway in animals. However, further research is needed, especially in plants to determine the reversible nature of this detoxification step.

Hydrolysis

Hydrolases, such as esterases, amidases, and *O-alkyl hydrolases associated with subcellular* fractions in the liver, in other organs, and in plasma play an important role in the degradation of many pesticides, including; OP insecticides, pyrethroid insecticides, phenoxy alkanoic acid herbicides, and anilide herbicides (*7, 10*).

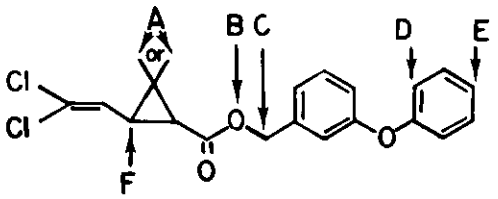
The insecticide, malathion, is hydrolyzed by the cleavage of one carboethoxy group in the rat *in vivo* and by a purified rat liver carboxylesterase *in vitro*. The hydrolysis product was identified by NMR spectroscopy as the α-monoacid (*55*) [Eq. (2)]. This detoxification pathway confers useful selectivity to this insecticide by virtue of its facile hydrolytic cleavage in mammalian species. The selective toxicity of malathion to insects is probably related to the levels of carboxylesterase in various species (*10*). Carboxylesterase activity was found to be high in malathion-resistant strains or species of insects (*55*). Little information is available on plant esterases hydrolyzing malathion (*10*). However, it is known that malathion is also hydrolyzed in plants (*7*).



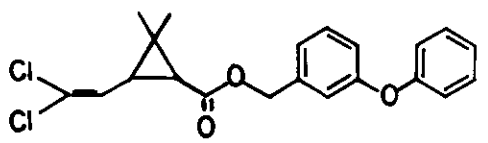
(2)

Hydrolysis also plays a key role in the mechanism of action of phenoxy acid derivatives in plants. In plants the nitrile group of ω-(2,4-dichlorophenoxy)alkane nitriles hydrolyzes and the resulting carboxyl group undergoes β-oxidation to form 2,4-dichlorophenoxyacetic acid (2,4-D) and other alkanoic acid derivatives (*57*). The selective tolerance of many plant species to these herbicides is governed by their rate of formation and degradation in plants. The herbicide propanil is hydrolyzed by rice aryl acylamidase to yield the nonherbicidal 3,4-dichloroaniline and propionic acid. However, the susceptible barnyardgrass, a major weed in rice fields, has a low titer of this enzyme and succumbs to the herbicide (*58*).

Photostable, synthetic pyrethroids are a new group of insecticides combining excellent insecticidal activity and low mammalian toxicity (*59*). Metabolism studies with these insecticides show that hydrolysis is their major degradative pathway and ease of hydrolysis parallels the loss of insecticidal



(+) trans-permethrin



(+) cis-permethrin

Table 5. Metabolic reactions for *trans*- and *cis*-permethrin in the rat, cow, and plants.[a]

Reactions	Site[b]	Rat (R) Cow (C) Plant (P)
Hydrolysis	B	R, C, P
Hydroxylation	E	R, C, P
Hydroxylation	D	R, P
Hydroxylation	A	R, C, P
Epimerization	F	P
Hydrolysis-oxidation	B, C	R, C, P
Hydroxylation-hydrolysis	E, B	R, C, P
	D, B	R, C, P
	A, B	R, C, P
Double hyroxylation	A, E	C

[a] Data of Okawa et al. (*60*) and Gaughan et al. (*61*).
[b] Letters denote reaction sites for the structure shown above this table.

activity.

Permethrin is one of the most active synthetic pyrethroids discovered to date. It has two geometric isomers (*cis, trans*), and each of these has two optical isomers. The letter notations in the structure indicate the sites of metabolic attack in the rat, cow, and plant based on studies by Ohkawa, et al. (*60*) and Gaughan, et al. (*61*). The types of reactions and organisms in which they occur are shown in Table 5. Following topical application to leaves of bean plants, permethrin was extensively hydrolyzed at the ester linkage (B) and hydroxylated on the phenoxy group (E, D) (*60*). Minor transformations in cotton and bean plants involved hydroxylation of a gem methyl group on the cyclopropane moiety (A) and photolytically induced epimerization of the *trans* and *cis* isomers (*61*). The *cis* isomers are more insecticidal and persist longer on plants under field conditions (*60*). These are interesting examples of differential transformations as a function of stereochemical configuration of the pesticide.

Rats and ruminants also metabolize permethrin, primarily by hydrolysis (B) and hydroxylation of a *gem*-methyl group (A) and to a lesser extent by aryl hydroxylation (D, E) (*61*). Stereospecificity of the molecule also affects metabolism in animals. The *trans* isomers were hydroxylated in the 4-position (E), while the *cis* isomers were hydroxylated in either the 2- (D) or 4- (E) positions. Conversely the *cis* isomers were hydroxylated in only one *gem*-methyl group (A) while the *trans* isomers were hydroxylated in either position (*61*). In animals, the cleaved metabolites were excreted without conjugation as glucuronides, glycine, and glutamic acid conjugates. Glycoside conjugates of hydroxy esters and the phenoxybenzyl metabolites were recovered from bean and cotton plants (*61*).

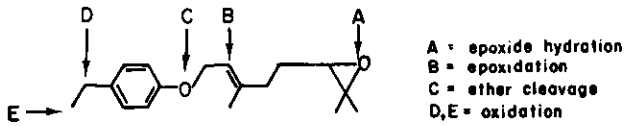
In *in vitro* studies using mouse liver microsomes it was demonstrated that hydrolysis of permethrin by esterases is the most important reaction in the degradation of permethrin (*62*). The ester cleavage reaction is NADPH dependent. Pretreatment of mice with an oxidase (piperonyl butoxide) or esterase (*S,S,S*-tributyl phosphorotrithioate) inhibitors increased the toxicity of several synthetic pyrethroids to mice (*62*).

Epoxidation and Epoxide Hydration

Epoxidation of the unchlorinated double bond is an important metabolic transformation of certain cyclodiene insecticides including, heptachlor, aldrin, isodrin, and chlordene (*63*). Epoxidation is a facile reaction occurring *in vitro* and *in vivo* in animals (*11*) and in plants (*7*). A number of these epoxides are equally or more insecticidal than the parent compounds (*7*). Arene and alkene epoxides may undergo hydration to the corresponding *trans*-diols in a reaction mediated enzymatically by an epoxide hydrase. While epoxide hydrases have been exhaustively studied and characterized in mammals and insects (*10*), considerably less information is available on this enzyme system in plants (*7*).

Several insect growth regulators (IGRs) are epoxidized terpenes and sesquiterpenes (*64*). Hydration of the epoxide usually reduces or destroys juvenilizing activity in insects (*65, 66*).

The metabolism of the IGR R-20458 in living rats was reported by Hoffman et al. (*67*) and in living



IGR, R-20458

mice and *in vitro* mammalian systems by Gill et al. (*68*). It was established in these studies that the IGR undergoes rapid metabolism and excretion. Based on identified urinary metabolites, epoxide hydration at site (A) is a major degradative reaction shown to occur *in vivo* (*67, 68*) and *in vitro* (*68*). In the presence of microsomal liver enzymes R-20458 was oxidized (site B) to diepoxide derivatives (*68*). The epoxidized species were hydrated in both *in vivo* and *in vitro* systems by reactions catalyzed by epoxide hydrases (*68*). Other metabolic reactions (sites C, D, E) also play a key role in the degradative biotransformation of this IGR in animals. Although the fate of this IGR was not determined in higher plants, Gill et al. (*68*) reported that algae, *Chlamydomonas* sp. readily hydrated the epoxide moiety and sparingly converted R-20458 to the diepoxide derivative.

Discussion

The foregoing discussion and selected examples of biotransformation reaction of pesticides delineate the ability of animals, including mammals, insects and higher plants to biotransform pesticides to which they are exposed either intentionally or unintentionally in the environment.

As shown in Tables 1 and 2, most of the biotransformation reactions are oxidative and mediated by microsomal enzymes. However, reductions and nonmicrosomal transformation also play a key role in the degradation of these xenobiotic chemicals.

In a number of instances, significant quantitative differences are evident in the products of biotransformation in animals and plants. Some of these differences can be attributed to the relatively shorter residence time of pesticides in animals. Animals, in part, due to an efficient circulatory and excretory system tend to eliminate biotransformation products primarily in urine and feces. Furthermore, contact with the pesticide is usually of short duration or of a transitory nature. Plants usually are in contact with the pesticide for longer periods of time, especially if they grow in a treated soil. Furthermore, due to a less efficient circulatory system and limited excretion, the pesticide may reside for a longer time in a plant. As a consequence of the longer residence time in the plant, certain pesticides such as OP insecticides have a greater opportunity to oxidize and remain in the plant as a desulfurated species (oxon) and/or as the sulfoxides and sulfones of those OP esters containing a thioether moiety. These activated metabolites are seldom encountered as significant terminal residues in animals *in vivo*.

Physiological, environmental, and edaphic factors, as well as dose, can also influence the biotransformation and ultimate disposition of pesticides and their degradation products in living organisms (*7, 69*). Furthermore, the possibility of multiple applications of heterogeneous pesticides may affect the metabolism of each component of the mixture and modify the response of the organism to the chemical(s) (*7*).

Metabolism studies are very useful in delineating the selective toxicity and mode of action of pesticides in target and nontarget organisms. They serve also as an integral component in assessing the safety of these chemicals to man and his environment.

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